Applicants also enclose a substitute specification with the pages renumbered starting with page 11, to incorporate the changes to the specification and to delete pages 38, 44, and 53, which were left blank due to the use of a page end before a table.

Respectfully submitted,

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October 24, 2001
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Response to Notice of Non-Compliant Amendment (37 C.F.R. § 1.121)

CERTIFICATE OF MAILING UNDER 37 C.F.R. § 1.8(a)

I hereby certify that this Response to Notice of Non-Compliant Amendment (37 C.F.R. § 1.121), and any paper referred to as being attached or enclosed, are being deposited with the United States Postal Service on the date shown below with sufficient postage as first-class mail in an envelope addressed to Commissioner for Patents, Washington, D.C. 20231.

Jean Hicks

Date: October 24, 2001

ATL1 #490006 v1

Version with markings to show changes made.

Autoantibodies" by the Board of Regents of the University of Oklahoma").

Figure 7 is a graph of the binding to the peptide GAGAGAGAGAGAGAGAGAGAGAGA (SEQ ID NO:7) from Epstein-Barr virus Nuclear Antigen-1 by lupus sera who all had anti-Sm and anti-nRNP precipitins, as compared to normal control sera.

Figures 8A-E are graphs of the binding to the overlapping octapeptides from Epstein-Barr virus Nuclear Antigen-1. Each octapeptide overlaps its neighbor by seven amino acids. Most of the glycine-alanine repeat has been omitted to avoid unnecessary redundancy. The binding of three controls are presented in Figures 8A, 8B and 8C and that of two lupus sera in Figures 8D and 8E. Figure 8A is from a normal who has no evidence of having been infected by Epstein-Barr virus by the assay for anti-Epstein-Barr virus Viral Capsid Antigen IgG. The other sera presented (Figures 8B through 8E) are all positive in this assay.

~~[Figure 9. Enrichment of phage recognized by anti PPPGMRPP (SEQ ID NO:4) antibodies. Affinity purified human anti-PPPGMRPP (SEQ ID NO:4) autoantibodies were used to screen $2X10^9$ PFU. After each enrichment step the number of bound phage was determined by plating out serial dilutions of the phage.~~

~~Figure 10 presents dot blot analysis of M13 phage mimotopes. $1X10^{10}$ pfu of each phage was vacuum transferred to nitrocellulose. Membranes were probed with affinity purified anti-PPPGMRPP (SEQ ID NO:4) antibodies. The graph shows the mean density as calculated using the NIH Image analysis software package. Peptides are 1. Wt M13, 2-GPPPGMRPP (SEQ ID NO:10), 3-SPLSTLL (SEQ ID NO:11), 4-KIGFPHI (SEQ ID NO:12), 5-IPRPLDY (SEQ ID NO:13), 6-MKLKHPP (SEQ ID NO:14), 7-ILPPPGY (SEQ ID NO:15), 8-AVIHRPP (SEQ ID NO:16), 9-ALIQRPP (SEQ ID NO:17), 10-VPLTVLL (SEQ ID NO:18), 11-SPPELK (SEQ ID NO:19), 12-KFLAPLQ (SEQ ID NO:20).~~

~~Figure 11 shows inhibition of anti-PPPGMRPP phage binding. Affinity purified anti-PPPGMRPP (SEQ ID NO:4) antibodies were pre-incubated with]~~

~~[100 ng of PPPGMRPP-MAP™ then incubated with 20,000 pfu of the indicated phage. Protein A agarose was used to isolate antibody-phage complexes. Phage were released by 0.1 M glycine ph=2.2. The amount of phage bound was determined by plating serial dilutions of the bound material. % inhibition=100X (1-# phage bound with 100 ng MAP/# phage bound with no MAP).]~~

Detailed Description of the Invention

In the United States, about 95% of the adult population has been, and continues to be, infected with Epstein-Barr virus. Observations described herein indicate a small proportion of these develope autoimmune disease, related to this virus. Other factors are also likely to be important in the development of autoimmune disease, but are not essential to understand in order to develop therapeutics and diagnostics for use in diagnosing, treating and preventing or ameliorating autoimmune diseases involving Epstein-Barr virus as the etiologic agent. Epstein-Barr virus is the probable etiologic agent for nearly all cases of lupus, which serves as an example of autoimmune disease.

Diagnostics and therapeutics derived from the discovery that Epstein-Barr virus causes autoimmune disease as applied to the prevention, diagnosis and treatment of autoimmune disease are described herein. Systemic lupus erythematosus (lupus) is the particular autoimmune disease evaluated and for which data have been obtained. Within lupus, the work on a molecular understanding of the relationship between anti-Sm and systemic lupus erythematosus and the relationship of anti-Sm autoantibodies to Epstein-Barr virus is the best illustration of the data supporting these diagnostics and therapeutics.

The experiments described herein to address Epstein-Barr virus in lupus were guided by the results of immunochemical studies, not by the previous studies. These data pointed toward a curious mechanism in the anti-Sm autoantibody system in lupus which could involve Epstein-Barr virus.

The technology applied to the problem is very important in two ways. First, the assays for anti-Epstein-Barr virus antibodies have been dramatically improved. The classic method is to evaluate antibody binding to an Epstein-

remaining phage stock was amplified by infecting *E. coli* cells, growing for 5 hours and recovery of the supernatant. The amplified phage stock was titered and a second round of enrichment was performed using $2x10^{11}$ pfu and protein-G agarose instead of protein-A agarose to capture antibody phage complex. This procedure was repeated 2 additional times for a total of 4 enrichments. Protein-A was alternated with protein-G agarose to avoid enriching peptides that bound to these proteins.

[~~Figure 9 shows a chart representing the titer of phage particles after each enrichment step.~~] During each round of enrichment a small population of phage that nonspecifically bind are also isolated. If only nonspecific binding clones were being isolated, the titer of the enriched phage should remain constant because the same amount of phage particles ($2 X 10^{11}$ pfu) were used in each enrichment step. The observed increase in phage particles isolated after each round of enrichment and amplification suggests that phage clones that specifically bound the anti-PPPGMRPP (SEQ ID NO:4) antibodies were being enriched.

Following the fourth round of amplification, 70 clones were isolated and sequenced (Table 9). Eleven distinct sequence motifs were identified. Both class I and class II motifs share obvious homology to PPPGMRPP (SEQ ID NO:4) peptide. It is interesting that these motifs correspond to either the N-terminal PPPG sequence or the C-terminal RPP sequence. No motifs were identified that represented the middle GMR sequence. This would suggest that the PPGMRPP may contain two distinct epitopes that are recognized by autoantibodies or that the critical region required for antibody recognition does not include the middle residues.

The current releases of GenBank and the Swiss protein databases were then searched with the peptide sequences obtained from the phage clones. Three of the peptide sequences were identical to proteins contained in these databases. The *E. coli* ornithine aminotransferase contains a peptide sequence that is identical to the type I peptide ILPPPGY (SEQ ID NO:15). Of the 70 clones sequenced, 9 of them contained sequences homologous to this sequence (two

Phage particles were obtained by PEG precipitation. The purified phage were titered by plating out serial dilutions on lawns of *E. coli*. Initially, Western blots were used to characterize the binding of anti-PPPGMRPP (SEQ ID NO:4) antibodies to the phage peptides. Equivalent amounts of phage particles (1×10^{10}) were separated on a 10% SDS gel and transferred to a nitrocellulose membrane. The results using anti-M13 pIII monoclonal antibody showed that there were approximately equivalent amounts of protein in each lane. When the purified anti-PPPGMRPP (SEQ ID NO:4) antibodies were used, differences in the intensity of the bands were observed. This would suggest that the antibodies have different affinities for the various peptides. It is also possible that denaturing the proteins in SDS caused structural epitopes to be lost. To investigate this possibility a dot blot system where phage were vacuum transferred to nitrocellulose under non-denaturing conditions [~~(Figure 10)~~] was used.

Intensities of the dots were measured using the NIH image analysis software package. The wild-type M13 clone had the lowest binding. Six of the clones had an apparent affinity lower than the GPPPGMRPP (SEQ ID NO:10) positive phage clone (Figure 9 dots, 3, 4, 5, 7, 9 and 10). Two of the clones had approximately equivalent affinity to the positive control (dots 6 and 11) while the remaining two clones showed significantly higher signals (dots 8 and 12). These results were verified by dilution experiments (data not shown).

In order to show specificity, two different inhibition experiments were set up. Aqueous phase inhibition experiments were performed by preincubating anti-PPPGMRPP (SEQ ID NO:4) antibodies with 0, 0.01, 0.1 or 1.0 g o f PPPGMRPP-MAP™. The antibodies were then incubated with 20,000 pfu of the GPPPGMRPP (SEQ ID NO:10) phage. Phage-antibody complexes were isolated using protein-A agarose. The amount of phage bound by the antibodies was determined by titering the bound and unbound material. Using 100 ng of PPPGMRPP-MAP™ blocked approximately 45% of the antibody-phage binding. (100 X (1- number of phage bound with 100ng MAP/number of phage bound without MAP). Similar experiments were then performed using six

different phage clones [~~(Figure 11)~~]. The PPPGMRPP-MAP™ inhibited binding to all six clones. However, the range of inhibition varied greatly (less than 10% to less than 70%). These results suggest that the antibody-phage binding is due to the sequence of the expressed peptides. However, the affinity of the antibodies for the phage clones varies significantly. The results obtained in these experiments were consistent with the results obtained in the dot blots. Interestingly, in each of these experiments the apparent affinity for the Epstein-Barr virus peptide SPPEWLK (SEQ ID NO:95) is higher than the GPPPMRPP (SEQ ID NO:97)-phage.

Example 11. Use of a vaccine composition designed to induce a response to prevent or treat an autoimmune disease.

Assuming Epstein-Barr virus causes autoimmune disease, then an effective vaccine which induces a protective response against Epstein-Barr virus has the potential to protect the host from the autoimmune disease. This is particularly true if the structure(s) which induce autoimmune disease is(are) altered or removed from the vaccine.

Once infected, this virus is latent and in most, perhaps virtually all, individuals the virus emerges from latency at a low level throughout the remainder of life. The viral infected cells or the immune response required to suppress the virus have the potential to be extremely important in inducing or sustaining the autoimmune disease. Cells latently infected by Epstein-Barr virus may also alter the immune response. Consequently, treatments designed to suppress or eliminate Epstein-Barr virus have the potential to ameliorate the symptoms and tissue damage of the autoimmune disease.

Treatments expected to be useful against autoimmune disease include compositions that suppress the emergence of Epstein-Barr virus from latency. Also, agents for gene therapy directed against Epstein-Barr virus will be useful. Biologicals may also prove useful against Epstein-Barr virus by altering the intracellular environment, making it less hospitable to the virus by directly affecting the virus or by making the immune response against the virus less prone to support an autoimmune disease process.